Chronic lithium administration in a mouse model for Krabbe disease

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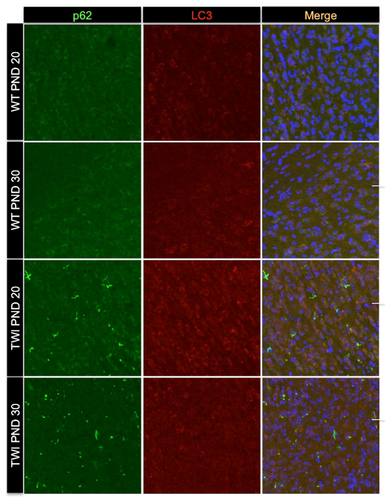
Introduction

Krabbe disease (KD; or globoid cell leukodystrophy) is an autosomal recessive lysosomal storage disorder caused by deficiency of the galactosylceramidase (GALC) enzyme. GALC lack causes the accumulation of the cytotoxic sphingolipid psychosine (PSY) in the central and peripheral nervous system (CNS and PNS), leading in the end to extensive demyelination and neurodegeneration. Unfortunately, no cure is currently available for KD. Clinical applied treatments are supportive only. Recently, we demonstrated that two differently acting autophagy inducers (lithium and rapamycin) can improve some KD hallmarks in-vitro, laying the foundation for their in-vivo pre-clinical testing. [Del Grosso et al., 2016; Del Grosso et al., 2019]

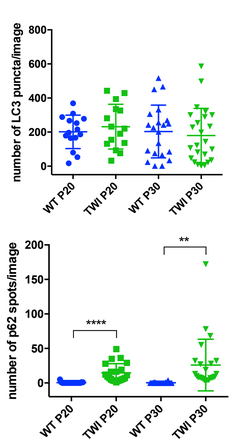
Methods

Here, we tested lithium carbonate in the spontaneous mouse model for KD, the Twitcher (TWI) mouse. The drug is administered ad libitum via drinking water (600 mg/L) starting from post natal day (PND) 20. We longitudinally monitor the mouse motor performance through the grip strength, the hanging wire and the rotarod tests, and a set of biochemical parameters related to the KD pathogenesis [i.e., GALC enzymatic activity, PSY accumulation and astrogliosis]. Additionally, we investigate the expression of some crucial markers related to the two pathways that could be altered by lithium: the autophagy and the β-catenin-dependent pathways.

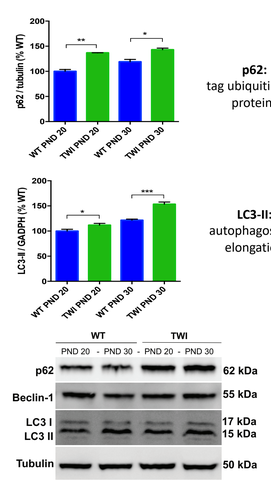
1. Autophagy dysregulation in Krabbe disease



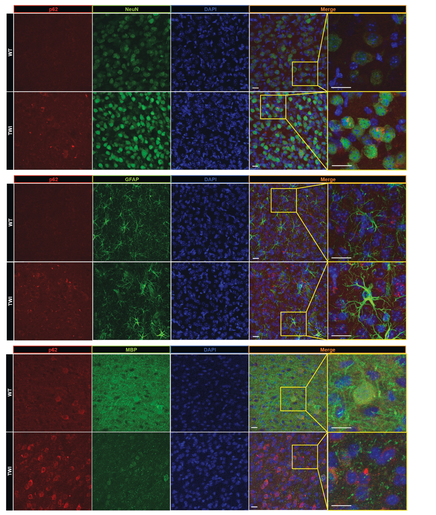
Immunohistochemistry staining of WT and TWI brain with anti-LC3 (red) and anti-p62 (green) antibodies (nuclei are stained with DAPI, blue). Scale bar: 37 μm.



Analysis of average number and area of the LC3 and p62 puncta per image. Mean ± SD; Student's t-test. N=2 mice for condition.



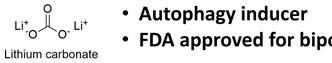
Western blot analysis of the total p62, LC3-II and Beclin-1 content in whole brain lysates. Mean ± SEM. Student's t-test. N ≥ 3.



Immunohistochemistry staining of WT and TWI brain with anti-p62 (green) and anti-NeuN/GFAP/MBP antibodies (nuclei are stained with DAPI, blue). Scale bar: 20 μm.

Del Grosso A. et al., *Neurobiology of disease*; 2019

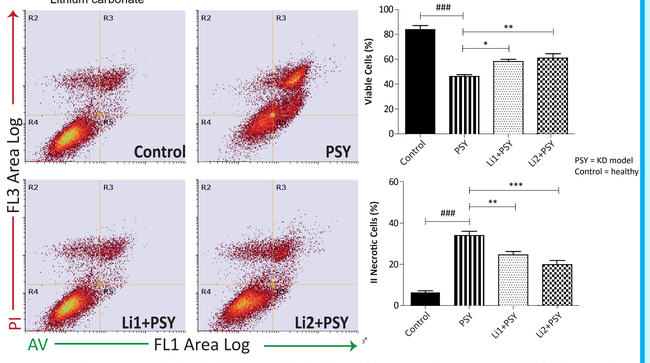
2. Lithium *in-vitro*



Lithium carbonate

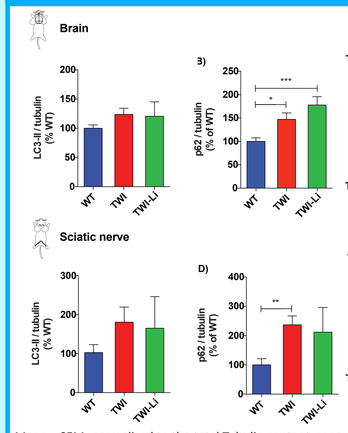
- **Autophagy inducer**
- **FDA approved for bipolar disorder**

Del Grosso A. et al., *J Neurosci Res*; 2016

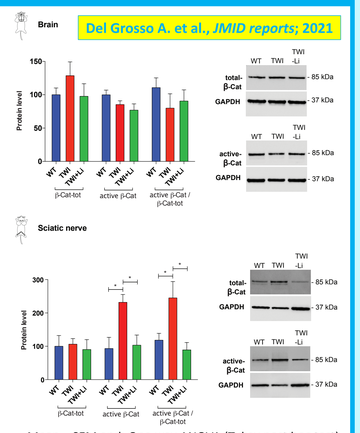


Lithium treatment improves cell viability in PSY-treated MO3.13 cells. Representative dot-plots of MO3.13 cells stained with annexin V/PI and analyzed by flow cytometry: R4, R5, R3 and R2 quadrants define, respectively, the healthy, apoptotic, secondary necrotic, and necrotic populations. Student's t-test. N = 4.

4. Lithium *in-vivo*



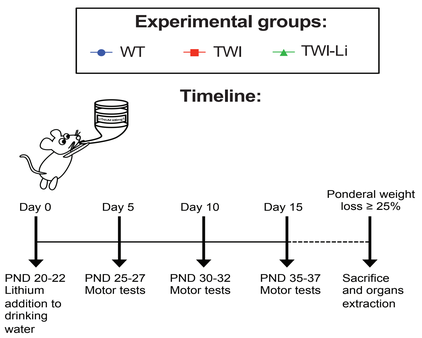
Mean ± SEM, normalized to the total Tubulin content, reported as percentage versus WT values; One way ANOVA (Tukey post hoc test). *N* = between 5 and 15 mice for each experimental group.

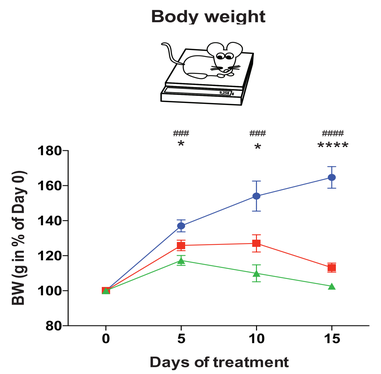


Mean ± SEM and; One way ANOVA (Tukey post hoc test). *N* = between 5 and 9 mice for experimental group.

Del Grosso A. et al., *JMID reports*; 2021

3. Lithium *in-vivo*

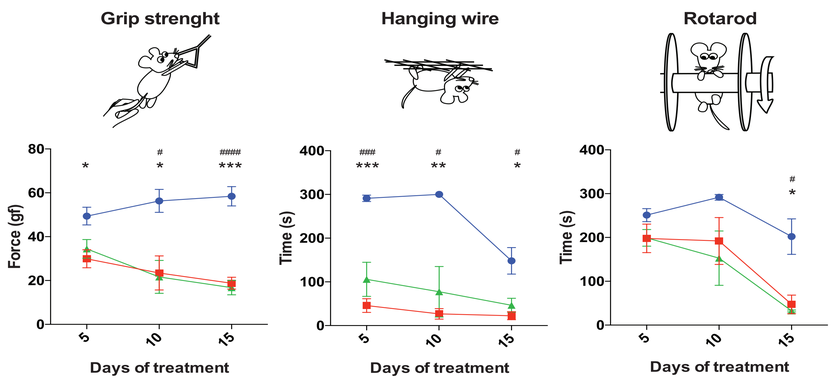


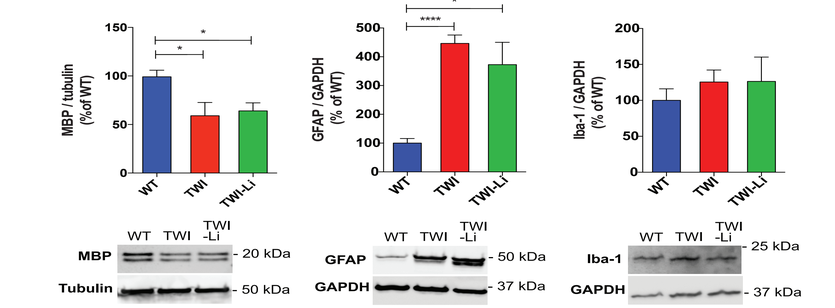


Administration of lithium carbonate by the drinking water in a mouse model of Globoid cell leukodystrophy.

Experimental design: Lithium carbonate (600 mg/L) has been administered via drinking water to the Twitcher (TWI) mice, starting at PND 20–22 (Day 0 of the experiments) and carry on until mice sacrifice (i.e., when ponderal weight loss ≥ 25%). As control, untreated Wilde type (WT) and TWI mice with similar age have been used for experiments. Motor tests (grip strength test, hanging wire test, and rotarod test) have been performed on treated (TWI-Li) and untreated (WT, TWI) mice every 5 days after Day 0: Day 5, Day 10 and Day 15.

Mean ± SEM and; One way ANOVA (Tukey post hoc test). *N* = between 3 and 10 mice for each experimental group.





Mean ± SEM, normalized to the total Tubulin or Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) content, reported as percentage versus WT values; One way ANOVA (Tukey post hoc test). *N* = between 5 and 9 mice for each experimental group.

Del Grosso A. et al., *JMID reports*; 2021

Conclusions

Lithium has not a significant rescue effect on the TWI phenotype, although it can slightly and transiently improve muscle strength. With this administration protocol, lithium is unable to stimulate autophagy in the TWI mice CNS, whereas results suggest that it can restore the β-catenin activation status in the TWI sciatic nerve. Overall, these data provide intriguing inputs for further evaluations of lithium treatment in TWI mice.